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OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

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Regulatory Action: Registration Review

CAS No.: 68424-85-1, 7173-51-5

Subject: **Special Studies.** Dietary Residues in Food from Treating Countertops with ADBAC and DDAC.

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EXECUTIVE SUMMARY:

MRID# 46870701

An LC/MS/MS method (Method GPL-MTH-057) was developed and validated by Golden Pacific Laboratories for determining residues of didecyl dimethyl ammonium chloride (DDAC) and C_{12}, C_{14}, and C_{16} alkyl dimethyl benzyl ammonium chloride (ADBAC) in slices of bread, apple and bologna. The method was developed for use in a study examining the transfer of residues from treated surfaces to representative food commodities.

For this method, residues of DDAC and ADBAC are extracted from slices of bread, apples and bologna by homogenization with acetonitrile/water/formic acid (70:30:0.016, v:v). Aliquots of the extract are filtered and diluted, and deuterated internal standards are added for each analyte. Residues are then analyzed by LC/MS/MS using a C18 column and a mobile phase gradient of water to acetonitrile, each containing 0.2% formic acid. A single ion transition is monitored for each analyte, and the internal standards are used for quantitation of each compound. The validated limits of quantitation (LOQs) for the four analytes are expressed in terms of µg compound per unit area, and are ~0.002-0.005 µg/cm^2 for bread, ~0.004-0.050 µg/cm^2 for apple, and ~0.004-0.053 µg/cm^2 for bologna.

The method was validated using control samples of each matrix fortified at the LOQ and 20x LOQ for each analyte in apple and bologna and for C_{16} ADBAC in bread. For validation of C_{12} and C_{14} ADBAC and DDAC in bread, samples were fortified at the LOQ, 10x LOQ and 200x LOQ. Seven samples were fortified and analyzed for each commodity and each analyte at each fortification level. Apparent residues of each analyte were <LOQ in all unfortified control samples, and adequate recoveries (70-120%) were obtained from fortified samples of each food commodity, with the exceptions of C_{12} ADBAC from bread at the lowest fortification level and C_{16} ADBAC from bread at both the LOQ and 20x LOQ fortification levels. With the above exceptions, average recoveries of each analyte from bread, apples, and bologna averaged 80-102%, with coefficients of variation (CVs) of 1-8%. Recoveries of C_{12} ADBAC from bread fortified at ~0.002 µg/cm^2 averaged 114% with a CV of 22%, and recoveries of C_{16} ADBAC from bread at both levels averaged 66 and 61%, but had low CVs (3 and 5%).

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the analytical method test data are classified as scientifically acceptable. The data indicate that Method GPL-MTH-057 is adequate for determining residues of DDAC and ADBAC (C_{12}, C_{14} and C_{16}) in bread, bologna and apples.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

DDAC and ADBAC are antimicrobials used in several types of applications, such as indoor and outdoor hard surfaces (e.g. walls, floors, tables, toilets and fixtures) eating utensils, laundry, carpets, agricultural tools and vehicles, egg shells, shoes, milking equipment and udders, humidifiers, medical instruments, human remains, ultrasonic tanks, reverse osmosis units and water storage tanks. There are also DDAC and ADBAC-containing products that are used in residential and commercial swimming pools, in aquatic areas such as decorative ponds and fountains, and in industrial process and water systems such as re-circulating cooling water systems, drilling muds and packer fluids, oil well injection and wastewater system. Additionally, DDAC and ADBAC-containing products are used for wood preservation.

The chemical structure and nomenclature of DDAC and ADBAC, and the physicochemical properties of the technical grade of DDAC and ADBAC are presented in Tables A.1 and A.2.

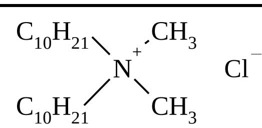
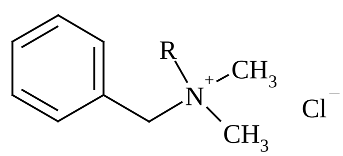
TABLE A.1. DDAC and ADBAC Nomenclature.	
Compound	
Common name	DDAC
Company experimental name	DDAC
IUPAC name	Didecyl dimethyl ammonium chloride
CAS name	Didecyl dimethyl ammonium chloride
CAS Registry number	7173-51-5
Compound	 R= C12, C14, C16
Common name	ADBAC (40% C_{12}, 50% C_{14}, and 10% C_{16})
IUPAC Name	n-Alkyl dimethyl benzyl ammonium chloride
CAS Name	n-Alkyl dimethyl benzyl ammonium chloride
CAS Registry number	68424-85-1

TABLE A.2. Physicochemical Properties of DDAC and ADBAC			
Parameter	Value (DDAC)	Value (ADBAC) [1]	Reference
Molecular weight	326.08	377.83	DDAC and ADBAC REDs (2006)
Melting point	228.81°C	241.02°C	
Density (at 25°C)	0.9216 g/cm^3	0.9429 g/cm^3	
Water solubility	Completely Soluble	Soluble	
Solvent solubility	Not stated	Soluble in Alcohols	
Vapor pressure (at 25°C)	2.33 x 10^{-11} mm Hg	3.53 x 10^{-12} mm Hg	

B. MATERIALS AND METHODS

Method GPL-MTH-057 is an HPLC/MS/MS method for determining residues of DDAC and ADBAC (C_{12}, C_{14} and C_{16}) in representative food commodities (apples, bread, and bologna). This method was developed by Golden Pacific Laboratories for use in a study designed to measure transfer of DDAC and ADBAC residues from treated laminate surfaces to food (see MRID 46870703).

B.1. Principle of the Method:

For this method, residues of DDAC and ADBAC (C_{12}, C_{14} and C_{16}) are extracted from slices of bread, apple and bologna by homogenization with acetonitrile/water/formic acid (70:30:0.016, v:v). Aliquots of the extracts from each sample are collected, filtered and diluted with either the extraction solution for analysis of DDAC or with acetonitrile/10mM ammonium formate (50:50 v:v) for analysis of ADBAC. Deuterated internal standards for DDAC and C_{12}, C_{14} and C_{16} ADBAC are added to the diluted extract and residues are then analyzed by HPLC/MS/MS (Table B.1). The HPLC system utilizes a reverse-phase C_{18} column with a mobile phase gradient of water to acetonitrile, each containing 0.2% formic acid. A single transition is monitored for each analyte and internal standard. Standard curves were generated for each analyte using the individual reference standards and deuterated reference standards.

TABLE B.1. Summary Parameters for the Analytical Method Used for the Quantitation of DDAC and ADBAC Residues in Bread, Apple and Bologna.

Method ID	GPL-MTH-057
Analyte(s)	DDAC, C_{12} ADBAC, C_{14} ADBAC, C_{16} ADBAC
Extraction solvent/technique	acetonitrile/water/formic acid (70:30:0.016, v:v).
Cleanup strategies	HPLC Phenomenex, Luna 3 µm, C18, 30x2.0 mm with mobile phase gradient of water to acetonitrile, each containing 0.2% formic acid.
Instrument/Detector	Sciex AP13000 LC/MS/MS with ESI interface, positive polarity. The following ion transitions were monitored for each analyte and internal standard: Analyte / Transition (m/z) / Internal Std. / Transition (m/z) DDAC / 326.28 → 186.15 / ^{3}d-DDAC / 329.28 → 189.15 C_{12}-ADBAC / 304.00 → 212.00 / ^{5}d-C_{12}-ADBAC / 309.00 → 212.00 C_{14}-ADBAC / 332.00 → 240.00 / ^{5}d-C_{14}-ADBAC / 337.00 → 240.00 C_{16}-ADBAC / 360.00 → 268.00 / ^{5}d-C_{16}-ADBAC / 365.00 → 268.00
Standardization method	Deuterated internal standard calibration curve.
Stability of std solutions	not reported
Retention times	Analyte / R_t DDAC / 1.42 min C_{12} ADBAC / 0.87 min C_{14} ADBAC / 1.21 min C_{16} ADBAC / 1.60 min

B.2 Method Validation

The above method was validated using control samples of apple (Red Delicious), Bread (Classic Wonder® Bread), and bologna (Oscar Mayer™ Beef Bologna). These foods were selected to represent a variety of types of food. For fortification, the slices of bread were cut to 10 x 10 cm squares. Apples were cut into thin slices (1/8" thick) and the slices were cut to form 10 x 10 cm pieces. The bologna slices were used without modification and had a diameter of 11 cm (95 cm^2).

The food samples were fortified with a diluted commercial formulation of SaniCare Lemon Quat, which is manufactured by Buckeye International and contains 2.54% DDAC and 1.69% ADBAC (40% C_{12}, 50% C_{14} and 10% C_{16}). For fortification, the commercial solution was diluted with ethanol to achieve target concentrations of approximately 1000, 50, and 5 µg/mL of DDAC. Solutions A and B were analyzed to determine the actual concentrations of each analyte in the fortifying solutions, and the concentration in Solution C was calculated based on dilution of Solution B (Table B.2).

The target LOQs of each analyte in each matrix are reported in Table B.2.2. Note that the fortification levels are expressed in terms of analyte mass per unit surface area (µg/cm^2) as the method was developed for a study evaluating the transfer of surface residues. Apple and bologna samples were fortified with the mixed standard solutions at the target LOQ and 20x LOQ. For bread, samples were fortified at the LOQ, 10x LOQ, and 200x LOQ for analysis of DDAC, C_{12} ADBAC, and C_{14} ADBAC, and at the LOQ and 20x LOQ for analysis of C_{16} ADBAC.

For fortification, samples of each food were placed on a piece of aluminum foil and fortified with 100 µL of the appropriate mixed standard solution. Samples were allowed to absorb the solution for ~ 1 minute and were then extracted and analyzed using the above LC/MS/MS method. For each type of food, 7 samples were fortified and analyzed at each fortification level along with 3 unfortified control samples.

Table B.2.1. Concentrations of DDAC and ADBAC in Solutions used for Fortification.

Solution	Concentration (µg/mL)			
	DDAC	ADBAC		
		C_{12}	C_{14}	C_{16}
Solution A	1006	326	375	71.9
Solution B	50.3	16.3	18.8	3.60
Solution C	5.03	1.63	1.88	0.360

TABLE B.2.2. Summary of Method Limits of Quantitation (LOQ) for DDAC and ADBC from Bread, Apple and Bologna.

Matrix	Analyte	LOQ (µg/cm^2) [1]
Bread	C_{12} ADBAC	0.00163
	C_{14} ADBAC	0.00188
	C_{16} ADBAC	0.00360
	DDAC	0.00503
Apple	C_{12} ADBAC	0.0163
	C_{14} ADBAC	0.0188
	C_{16} ADBAC	0.00360

	DDAC	0.0503
Bologna	C_{12} ADBAC	0.0172
	C_{14} ADBAC	0.0198
	C_{16} ADBAC	0.00379
	DDAC	0.0529

1 As the method is being developed to study surface transfer of residues, the fortification levels are expressed in terms of mass/unit area.

C. RESULTS AND DISCUSSION

The recoveries of DDAC and ADBAC from fortified samples of bread, apple and bologna are reported in Table C.1. For bread, acceptable recoveries of all four analytes were obtained, although recoveries of C_{12} ADBAC were generally high (>110%) at the lowest fortification level (~0.002 µg/cm^2) and recoveries of C_{16} ADBAC were consistently low at both fortification levels (<70%). At the lowest fortification level (0.0016 µg/cm^2), recoveries of C_{12} ADBAC from bread averaged 114% with a CV of 22; however, recoveries from the 0.016 and 0.33 µg/cm^2 levels averaged 102 and 96%, with CVs of 3-4%. Average recoveries of C_{16} ADBAC from bread at both fortified levels were low (66 and 61%), but with CVs 3 and 5%; therefore, the method was considered to be acceptable for data collection. Adequate recoveries of C_{14} ADBAC and DDAC were obtained from bread at each fortification level (80-104%), and averaged 83-94% for C_{14} ADBAC and 89-95% for DDAC, with CVs of 1-7%.

For apple, adequate recoveries of each analyte were obtained at each fortification level (78-98%). Average recoveries for the four analytes from apple slices at each level were 80-87%, with CVs of 2-8%. Adequate recoveries of each analyte were also obtained from bologna at each fortification level (86-110%). Average recoveries for all four analytes from bologna at each level were 88-101%, with CVs of 3-8%. The validated LOQs for each analyte in each food commodity are reported in Table B.2.2.

No interferences were detected in the analyses. Apparent residues of each analyte were <LOQ in all control samples of bread, apple and bologna. Adequate sample calculations were provided, along with example chromatograms.

TABLE C.1. Validation of LC/MS/MS method (GPL-MTH-057) for Recovery of DDAC and ADBAC (C_{12}, C_{14} and C_{16}) from Fortified Samples of Bread, Apple and Bologna Slices. [1]

Matrix	Analyte	Spiking Level (µg/cm^2)	n	Recoveries Obtained (%)	Mean Recovery ± Std. Dev. (CV)
Bread	C_{12} ADBAC	0.00163	7	110, 120, 141, 69.9, 112, 132, **271** [2]	114 ± 25 (22)
		0.0163	7	96.9, 106, 101, 97.5, 102, 104, 107	102 ± 4 (4)
		0.326	7	92.3, 99.1, 95.4, 96.6, 95.1, 99.4, 93.9	96 ± 3 (3)
	C_{14} ADBAC	0.00188	7	89.4, 94.1, 96.3, 84.6, 98.9, 93.6, 104	94 ± 6 (7)
		0.0188	7	84.0, 82.4, 81.9, 84.6, 82.4, 82.4, 84.0	83 ± 1 (1)
		0.375	7	79.7, 82.1, 84.0, 88.0, 81.6, 83.7, 84.3	83 ± 3 (3)
	C_{16} ADBAC	0.00360	7	68.6, 61.7, 64.7, 63.3, 70.6, 64.7, 67.2	66 ± 3 (5)
		0.0719	7	62.9, 57.9, 60.2, 61.6, 59.2, 63.4, 63.0	61 ± 2 (3)
	DDAC	1.01	7	91.8, 90.1, 91.8, 89.5, 88.9, 90.7, 95.4	91 ± 2 (2)
		0.0503	7	95.4, 102, 95.8, 102, 95.6, 89.3, 87.1	95 ± 6 (6)
		0.00503	7	89.6, 95.5, 82.2, 90.5, 82.2, 89.3, 93.3	89 ± 5 (6)
Apple	C_{12} ADBAC	0.0163	7	84.0, 79.8, 82.8, 82.2, 85.3, 81.0, 82.2	83 ± 2 (2)
		32.6	7	81.3, 84.4, 81.3, 78.5, 85.0, 80.1, 85.9	82 ± 3 (3)
	C_{14} ADBAC	0.0188	7	81.9, 79.3, 79.3, 85.1, 84.0, 82.4, 81.9	82 ± 2 (3)
		0.375	7	81.1, 82.4, 80.5, 84.8, 97.9, 80.0, 88.5	85 ± 6 (8)
	C_{16} ADBAC	0.00360	7	88.1, 93.9, 83.1, 81.1, 84.7, 78.6, 81.1	84 ± 5 (6)
		0.0719	7	86.5, 83.7, 87.5, 83.3, 96.4, 83.7, 90.3	87 ± 5 (5)
	DDAC	0.0503	7	82.5, 80.1, 81.9, 77.9, 81.5, 79.1, 77.7	80 ± 2 (2)
		1.01	7	84.0, 84.1, 81.6, 79.2, 86.6, 89.3, 89.6	85 ± 4 (5)
Bologna	C_{12} ADBAC	0.0172	7	101, 91.9, 99.4, 90.1, 100, 102, 110	99 ± 7 (7)
		0.343	7	101, 99.4, 94.5, 98.5, 94.2, 109, 110	101 ± 6 (6)
	C_{14} ADBAC	0.0198	7	94.4, 97.0, 96.5, 91.9, 100, 100, 101	97 ± 3 (3)
		0.395	7	97.7, 96.5, 85.8, 90.6, 97.7, 99.2, 101	96 ± 5 (6)
	C_{16} ADBAC	0.00379	7	82.1, 89.4, 88.7, 82.6, 101, 96.3, 96.3	91 ± 7 (8)
		0.0757	7	86.7, 87.6, 89.4, 89.4, 94.6, 85.6, 94.6	90 ± 4 (4)
	DDAC	0.0529	7	90.2, 93.6, 96.2, 92.2, 95.7, 98.5, 101	95 ± 4 (4)
		1.06	7	95.3, 88.5, 89.7, 86.1, 86.3, 89.3, 86.6	89 ± 3 (4)

1 Fortifying standards were prepared in ethanol.
2 Value was not included in mean recovery.
CV = Coefficient of Variance

TABLE C.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of C_{12}, C_{14} and C_{16}ADBAC and DDAC Residues in Bread, Apple and Bologna.	
Analyte(s)	DDAC, C_{12} ADBAC, C_{14} ADBAC AND C_{16} ADBAC
Equipment ID	Sciex AP13000 LC/MS/MS with ESI interface, positive polarity
Limit of quantitation (LOQ)	LOQ was determined to be lowest level of standard added for each matrix, and is reported in Table B.2.2.
Limit of detection (LOD)	LOD was not reported
Accuracy/Precision	Recoveries were generally within the acceptable 70-120% range, with the exception of C_{16} ADBAC from bread at both fortification levels and C_{12} ADBAC from bread at the lowest fortification level. Recoveries of C_{12} ADBAC from bread at the 0.0016 µg/cm^2 fortification level averaged 114% with a CV of 22%, and recoveries of C_{16} ADBAC from bread at both levels averaged 66 and 61%, but had low CVs (3 and 5%). With the above exceptions for bread, average recoveries of each analyte from bread, apples, and bologna were 80-102%, with CVs of 1-8%.
Reliability of the Method	The low CV values obtained for each analyte at all but one fortification level (C12 ADBAC from bread) indicates that the method is reliable.
Linearity	The method/detector response was linear (coefficient of determination, $r^2 \leq 09998$) within the range of 0.1-3.0 ng/ml for DDAC $r^2 \leq$ 1.00 within 0.510-12.3 ng/ml for C_{12} $r^2 \leq 0.9998$ within 0.126-15.1 ng/ml for C_{14} $r^2 \leq 0.9996$ within 0.135-3.24 ng/ml for C_{16}
Specificity	No control chromatograms were provided. Chromatograms of standards contain shoulders which are consistent and do not appear to affect recovery. Peaks were well defined but occasionally asymmetrical. There appeared to be no carryover to the following chromatograms.

D. CONCLUSION

The LC/MS/MS Method GPL-MTH-057 is adequate for determining residues of DDAC and ADBAC (C_{12}, C_{14} and C_{16}) in bread, bologna and apples. The validated method LOQs for the four analytes are ~0.002-0.005 µg/cm^2 in bread, ~0.004-0.050 µg/cm^2 in apple, and ~0.004-0.053 µg/cm^2 in bologna.

E. REFERENCES

None

EXECUTIVE SUMMARY:

MRID# 46870702

A LC/MS/MS method (Method GPL-MTH-056) was developed and validated by Golden Pacific Laboratories, LLC for determining residues of didecyl dimethyl ammonium chloride (DDAC) and C_{12}, C_{14} and C_{16} alkyl dimethyl benzyl ammonium chloride (ADBAC) in aliquots of 50% isopropanol(IPA)/water, 10% ethanol/water and corn oil and in samples of dressing sponges and cotton percale sheets. The method was developed for use in studies examining the transfer of residues from treated surfaces to representative food commodities, hands, and percale.

For this method, residues of DDAC and ADBAC were extracted from dressing sponges and percale sheets by shaking samples with acetonitrile/water/formic acid (70:30:0.016, v:v). Residues were extracted from the 50% IPA and 10% ethanol solutions by dilution with acetonitrile/water/formic acid (70:30:0.016, v:v), and residues were extracted from corn oil samples by partitioning residues into acetonitrile, containing 0.2% formic acid. Aliquots of the extracts were filtered, diluted, and deuterated internal standards were added for each analyte. Residues were analyzed by LC/MS/MS using a C18 column and a mobile phase gradient of water to acetonitrile, each containing 0.2% formic acid. A single ion transition was monitored for each analyte, and the internal standards were used for quantitation of each compound. The validated method limits of quantitation (LOQs) for the four analytes were 0.93-13.2 ng/mL from the 50% IPA solution, 4.93-70.5 ng/mL from the 10% ethanol solution, 1.05-14.0 ng/mL from the corn oil, 0.37-5.29 µg/sample from the dressing sponges, and 44-622 µg/sample from the sheets of percale.

The above method was validated using replicate (n=7) control samples of each matrix at two fortification levels (LOQ and 10x or 20x LOQ). With the exception of only three samples, recoveries from the fortified samples were within the acceptable 70-120% range. Average recoveries (with coefficient of variation, CV) for all four analytes were 84-104% (CV, 1-7%) from the 50% IPA solution, 73-99% (CV, 4-15%) from the 10% ethanol solution, 83-99% (CV, 4-17%) from the corn oil, 92-102% (CV, 2-9%) from the dressing sponges, and 95-106% (CVs, 2-4%) from the cotton percale.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the analytical method test data are classified as scientifically acceptable. The data indicate that Method GPL-MTH-056 is adequate for quantitatively recovering residues of DDAC and ADBAC (C_{12}, C_{14} and C_{16}) from several types of solutions, dressing sponges and cotton percale used to recover residues from treated surfaces or hands.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

DDAC and ADBAC are antimicrobials used in several types of applications, such as indoor and outdoor hard surfaces (e.g. walls, floors, tables, toilets and fixtures) eating utensils, laundry, carpets, agricultural tools and vehicles, egg shells, shoes, milking equipment and udders, humidifiers, medical instruments, human remains, ultrasonic tanks, reverse osmosis units and water storage tanks. There are also DDAC and ADBAC-containing products that are used in residential and commercial swimming pools, in aquatic areas such as decorative ponds and fountains, and in industrial process and water systems such as re-circulating cooling water systems, drilling muds and packer fluids, oil well injection and wastewater system. Additionally, DDAC and ADBAC-containing products are used for wood preservation.

The chemical structure and nomenclature of DDAC and ADBAC, and the physicochemical properties of the technical grade of DDAC and ADBAC are presented in Tables A.1 and A.2.

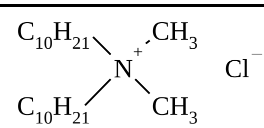
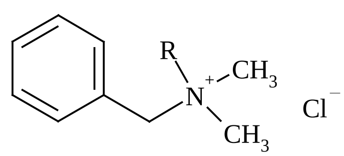
TABLE A.1. DDAC and ADBAC Nomenclature.	
Compound	
Common name	DDAC
Company experimental name	DDAC
IUPAC name	Didecyl dimethyl ammonium chloride
CAS name	Didecyl dimethyl ammonium chloride
CAS Registry number	7173-51-5
Compound	 R= C12, C14, C16
Common name	ADBAC (40% C_{12}, 50% C_{14}, and 10% C_{16})
IUPAC Name	n-Alkyl dimethyl benzyl ammonium chloride
CAS Name	n-Alkyl dimethyl benzyl ammonium chloride
CAS Registry number	68424-85-1

TABLE A.2. Physicochemical Properties of DDAC and ADBAC			
Parameter	Value (DDAC)	Value (ADBAC)	Reference
Molecular weight (g/mol)	326.08	377.83	DDAC and ADBAC REDs (2006)
Melting point	228.81°C	241.02°C	
Density (at 25°C)	0.9216 g/cm^3	0.9429 g/cm^3	
Water solubility	Completely Soluble	Soluble	
Solvent solubility	Not stated	Soluble in Alcohols	
Vapor pressure (at 25°C)	2.33 x 10^{-11} mm Hg	3.53 x 10^{-12} mm Hg	

B. MATERIALS AND METHODS

Method GPL-MTH-056 entitled, "Analytical Method for the Determination of Didecyl Dimethyl Ammonium Chloride (DDAC) and C_{12}, C_{14} and C_{16} Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) is a HPLC/MS/MS method for determining residues of DDAC and ADBAC (C_{12}, C_{14} and C_{16}) in solutions used for quantitatively recovering these compounds from treated surfaces and hands, and materials used to clean surfaces and hands following treatment (i.e. dressing sponges and percale sheets). This method was developed by Golden Pacific Laboratories, LLC for use in a study designed to measure transfer of DDAC and ADBAC residues from treated laminate surfaces to food and/or hands (see MRIDs 46870703 and 46870704).

B.1.1. Principle of the Method:

For this method, residues of DDAC and ADBAC (C_{12}, C_{14} and C_{16}) were extracted from dressing sponges and percale by shaking these samples for 15 minutes in acetonitrile/water/formic acid (70:30:0.016, v:v). Residues were extracted from the 50% IPA and 10% ethanol solutions by diluting the samples with acetonitrile/water/formic acid (70:30:0.016, v:v), and residues were extracted from corn oil samples by partitioning residues into acetonitrile, containing 0.2% formic acid.

Aliquots of the extracts from each sample were collected, filtered and diluted with either the extraction solution for analysis of DDAC or with acetonitrile/10mM ammonium formate (50:50 v:v) for analysis of ADBAC. Deuterated internal standards for DDAC and C_{12}, C_{14} and C_{16} ADBAC were added to the diluted extracts, and residues were analyzed by HPLC/MS/MS (Table B.1). The HPLC system utilizes a reverse-phase C_{18} column with a mobile phase gradient of water to acetonitrile, each containing 0.2% formic acid. A single transition ion was monitored for each analyte and internal standard. Standard curves were generated for each analyte using the individual reference standards and deuterated reference standards.

TABLE B.1. Summary Parameters for the Analytical Method Used for the Quantitation of DDAC and ADBAC Residues from Cleaning and Rinse Solutions and Dressing Sponges and Percale.	
Method ID	GPL-MTH-057
Analyte(s)	DDAC, C_{12} ADBAC, C_{14} ADBAC, C_{16} ADBAC
Extraction solvent/technique	acetonitrile/water/formic acid (70:30:0.016, v:v).
Cleanup strategies	HPLC Phenomenex, Luna 3 µm, C18, 30x2.0 mm with mobile phase gradient of water to acetonitrile, each containing 0.2% formic acid.
Instrument/Detector	Sciex AP13000 LC/MS/MS with ESI interface, positive polarity. The following ion transitions were monitored for each analyte and internal standard: Analyte / Transition (m/z) / Internal Std. / Transition (m/z) DDAC / 326.28 → 186.15 / DDAC-d_3 / 329.28 → 189.15 C_{12}-ADBAC / 304.00 → 212.00 / C_{12}-ADBAC-d_5 / 309.00 → 212.00 C_{14}-ADBAC / 332.00 → 240.00 / C_{14}-ADBAC-d_5 / 337.00 → 240.00 C_{16}-ADBAC / 360.00 → 268.00 / C_{16}-ADBAC-d_5 / 365.00 → 268.00
Standardization method	Deuterated internal standard calibration curve.
Stability of std solutions	Not reported
Retention times	Analyte / R_t DDAC / ~1.45 min C_{12} ADBAC / ~0.83 min C_{14} ADBAC / ~1.26 min C_{16} ADBAC / ~1.63 min

B.2 Method Validation

The above method was validated using control samples of dressing sponges, corn oil, 50% IPA in water, 10% ethanol in water, and percale fortified with known amounts of DDAC and ADBAC percale. These matrices were selected as they were utilized in related studies examining the transfer of DDAC and ADBAC residues to food, hands and fabric from treated laminate surfaces.

The different samples were fortified with a diluted commercial formulation of SaniCare Lemon Quat, which is manufactured by Buckeye International and contains 2.54% DDAC and 1.69% ADBAC (40% C_{12}, 50% C_{14} and 10% C_{16}). For fortification, the commercial solution was diluted with ethanol to achieve target concentrations of approximately 1000, 50, and 10 µg/mL of DDAC. The solutions were then analyzed to determine the actual concentrations of each analyte in the fortifying solutions (Table B.2).

The target LOQs of each analyte in each matrix are reported in Table B.2.2. Note that the fortification levels for the liquids are expressed in terms of ng/mL and the fortification levels for the dressing sponges and percale sheets are reported in terms of µg /sample. All samples except the corn oil were fortified with the mixed standard solutions at the target concentrations of the LOQ and 20x LOQ. Corn oil samples were fortified at the LOQ and 10x the LOQ.

For fortification of the dressing sponges, two wipes were pre-moistened with 5 mL of 50% IPA in water and then fortified with 100µL of the appropriate solutions (A and B). The fortified samples were then allowed to sit for 1 minute prior to extraction. For the percale, a 27.5 x 39.5 inch sheet of cotton percale was fortified with 0.5-0.6 mL of the appropriate solutions and also allowed to sit for 1 minute prior to extraction. Samples of the dressing sponges and percale were then extracted and analyzed as described above.

For the liquid samples, aliquots of 50% IPA/water (400 mL), 10% ethanol/water (75 mL), and corn oil (75 mL) were fortified with 100-200 µL of the appropriate solutions and then shaken for 10 seconds to mix the samples. The fortified samples were extracted and analyzed as described above.

Seven replicate samples were fortified and analyzed at each fortification level for each matrix, along with three unfortified control samples.

Table B.2.1. Concentrations of DDAC and ADBAC in Solutions used for Fortification.

Solution	Concentration (µg/mL)			
	DDAC	ADBAC		
		C_{12}	C_{14}	C_{16}
Solution A	1037	336	386	74.0
Solution B	52.9	17.0	19.5	3.70
Solution C	10.5	3.44	3.98	0.791

TABLE B.2.2. Summary of Limits of Quantitation (LOQs) for DDAC and ADBAC from Dressing Sponges, 50% Isopropanol in Water, 10% Ethanol in Water, Corn Oil and Cotton Percale.

Matrix	Method LOQ [1]			
	C_{12} ADBAC	C_{14} ADBAC	C_{16} ADBAC	DDAC
50% isopropanol/water	4.25 ng/mL	4.88 ng/mL	0.925 ng/mL	13.2 ng/mL
10% ethanol/water	22.7 ng/mL	26.0 ng/mL	4.93 ng/mL	70.5 ng/mL
Dressing Sponges	1.70 µg/sample	1.95 µg/sample	0.370 µg/sample	5.29 µg/sample
Corn Oil	4.59 ng/mL	5.30 ng/mL	1.05 ng/mL	14.0 ng/mL
Cotton Percale	202 µg/sample	232 µg/sample	44.4 µg/sample	622 µg/sample

[1]The method LOQ for each analyte is expressed in terms of ng/mL for the solutions and in terms of µg/samples for the dressing sponges and cotton percale.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

The recoveries of DDAC and ADBAC from samples of each matrix fortified at the LOQ and at 10x or 20x the LOQ are reported in Table C.1. Recoveries of all four analytes were adequate (70-120%) from all samples with the exception of three samples of the 10% ethanol solution fortified with C_{16} ADBAC at the LOQ (62%) and fortified with DDAC at the LOQ (59% and 57%).

For the 50% IPA/water solution, recoveries of DDAC, C_{12} and C_{14} ADBAC averaged 98-103%, with CVs of 1-3%. Recoveries of C_{16} ADBAC from the 50% IPA were slightly lower, averaging

84% and 99% at the two fortification levels with CVs of 6% and 7%, respectively. Recoveries from the 10% ethanol solution averaged 73-99%, and were also more variable with CVs of 4-15%. For both the dressing sponge and cotton percale samples, recoveries of all four analytes averaged 92-106%, with CVs of 2-9%. For corn oil, recoveries of all four analytes average 83-100%, but were somewhat more variable with CVs of 4-17%.

No interferences were detected in the analyses. Apparent residues of each analyte were <LOQ in all control samples of each matrix. Adequate sample calculations were provided. Example chromatograms were also provided with the exception of blank control samples.

TABLE C.1. Recovery Results from Method Validation of Dressing Sponges, 50% Isopropanol in Water, 10% Ethanol in Water, Corn Oil or Percale using the Data-Gathering Analytical Method. [1]					
Matrix	Analyte	Spiking Level	n	Recoveries Obtained (%) [2]	Mean Recovery ± Std. Dev. (CV)
50% IPA/water	C_{12} ADBAC	4.25 ng/mL	7	101, 102, 103, 98..8, 99.8, 105, 102	102 ± 2 (2)
		84.0 ng/mL	7	101, 99.8, 106, 107, 97.4, 100, 103	102 ± 3 (3)
	C_{14} ADBAC	4.88 ng/mL	7	100, 100, 100, 105, 106, 101, 106	103 ± 3 (3)
		96.5 ng/mL	7	105, 103, 106, 106, 102, 101, 105	104 ± 2 (2)
	C_{16} ADBAC	0.925 ng/mL	7	91.0, 83.5, 77.8, 85.4, 79.1, 80.2, 89.1	84 ± 5 (6)
		18.5 ng/mL	7	101, 110, 102, 97.3, 89.2, 101, 93.5	99 ± 7 (7)
	DDAC	13.2 ng/mL	7	100, 101, 103, 100, 100, 102, 99.2	101 ± 1 (1)
		259 ng/mL	7	97.3, 95.4, 98.5, 103, 98.8, 97.3, 95.0	98 ± 3 (3)
10% ethanol/water	C_{12} ADBAC	22.7 ng/mL	7	92.5,[3] 102, 94.7, 94.7, 90.7, 91.6, 85.0	93 ± 5 (6)
		448 ng/mL	7	99.8, 102, 93.8, 94.6, 98.0, 94.4, 87.5	96 ± 5 (5)
	C_{14} ADBAC	26.0 ng/mL	7	75.0, 101, 100, 92.7, 89.6, 93.8, 78.1	90 ± 10 (11)
		515 ng/mL	7	98.1, 106, 99.0, 96.3, 99.0, 102, 95.7	99 ± 4 (4)
	C_{16} ADBAC	4.93 ng/mL	7	**61.7,** 89.2, 76.1, 73.4, 86.8, 86.8, 90.9	81 ± 11 (13)
		98.7 ng/mL	7	84.0, 92.1, 89.2, 82.2, 81.5, 90.9, 78.0	85 ± 5 (6)
	DDAC	70.5 ng/mL	7	**58.7,** 81.4, 79.6, 82.4, 74.8, 79.3, **57.1**	73 ± 11 (15)
		1383 ng/mL	7	100, 101, 99.3, 97.9, 95.0, 96.3, 98.3	98 ± 2 (2)
Dressing Sponges	C_{12} ADBAC	1.70 µg/sample	7	92.9, 90.6, 92.9, 97.6, 92.4, 98.8, 94.1	94 ± 3 (3)
		33.6 µg/sample	7	91.4, 94.0, 87.8, 94.6, 96.1, 94.9, 95.5	94 ± 3 (3)
	C_{14} ADBAC	1.95 µg/sample	7	101, 99.5, 94.9, 102, 95.4, 104, 101	100 ± 3 (3)
		38.6 µg/sample	7	105, 107, 108, 102, 94.6, 93.5, 105	102 ± 6 (6)
	C_{16} ADBAC	0.370 µg/sample	7	91.1, 100, 110, 100, 107, 98.9, 103	101 ± 6 (6)
		7.40 µg/sample	7	102, 95.0, 89.2, 98.6, 87.8, 77.6, 91.8	92 ± 8 (9)
	DDAC	5.29 µg/sample	7	99.2, 100, 101, 102, 100, 105, 99.4	101 ± 2 (2)
		104 µg/sample	7	102, 94.5, 102, 104, 106, 100, 101	101 ± 4 (4)
Corn Oil	C_{12} ADBAC	4.59 ng/mL	7	89.8, 85.8, 87.4, 91.5, 92.2, 89.9,[3] 79.5	88 ± 4 (5)
		45.3 ng/mL	7	116, 108, 95.8, 94.3, 88.1, 95.6, 95.8	99 ± 10 (10)
	C_{14} ADBAC	5.3 ng/mL	7	95.1, 97.9, 95.8, 87.5, 95.8, 72.8, 107	93 ± 11 (11)
		52 ng/mL	7	106, 98.3, 101, 96.2, 98.8, 98.8, 94.2	99 ± 4 (4)
	C_{16} ADBAC	1.05 ng/mL	7	75.0, 81.6, 106, 81.6, 97.1, 73.6,[3] 66.1[3]	83 ± 14 (17)
		9.87 ng/mL	7	103, 96.1, 95.6, 107, 92.2, 92.8, 103	99 ± 6 (6)
	DDAC	14.0 ng/mL	7	98.6, 96.4, 99.3, 98.6, 95.7, 88.6, 78.6	94 ± 8 (8)
		141 ng/mL	7	101, 93.6, 104, 96.5, 101, 103, 99.3	100 ± 4 (4)
Cotton Percale	C_{12} ADBAC	202 µg/sample	7	107, 108, 107, 105, 103, 111, 104	106 ± 3 (3)
		4367 µg/sample	7	98.9, 102, 101, 103, 101, 101, 107	102 ± 3 (2)
	C_{14} ADBAC	232 µg/sample	7	105, 104, 107, 108, 104, 107, 108	106 ± 2 (2)
		5019 µg/sample	7	106, 101, 107, 108, 108, 107, 107	106 ± 2 (2)
	C_{16} ADBAC	44.4 µg/sample	7	92.8, 95.7, 97.5, 94.1, 91.9, 102, 94.4	96 ± 3 (4)
		962 µg/sample	7	107, 98.4, 102, 95.7, 99.7, 94.8, 94.8	99 ± 4 (5)
	DDAC	622 µg/sample	7	104, 103, 103, 98.2, 101, 105, 100	102 ± 2 (2)
		13468 µg/sample	7	92.6, 92.6, 103, 92.6, 93.6, 98.5, 96.8	96 ± 4 (4)

[1] Standards for fortification were prepared in ethanol.
[2] Recovery values outside the 70-120% range are indicated in **bold.**
[3] Value is an average of two analyses.

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of C_{12}, C_{14}, and C_{16} ADBAC and DDAC Residues in Dressing Sponges, Corn Oil , 50% isopropanol/water, 10% Ethanol/water and Percale.	
Analyte(s)	DDAC, C_{12} ADBAC, C_{14} ADBAC AND C_{16} ADBAC
Equipment ID	Sciex AP13000 LC/MS/MS with ESI interface, positive polarity
Limit of quantitation (LOQ)	LOQ was determined to be lowest level of standard added for each matrix, and is reported in Table B.2.2.
Limit of detection (LOD)	LOD was not reported
Accuracy/Precision	Recoveries were within the acceptable 70-120% range, with the exception of several samples of 10% ethanol fortified with either C16 ADBAC (62%) or DDAC (59% and 57%). Overall recoveries of all four analytes from each matrix, averaged 73-106% at both fortification levels, and the CVs for each analyte were 1-17%. CVs were >10% for various analytes at only three fortification levels for the 10% ethanol solution and in corn oil.
Reliability of the Method	The low CV values (<10%) obtained for each analyte at all but a few fortification levels indicate that the method is reliable.
Linearity	The method/detector response was linear (coefficient of determination, $r^2 \leq 0.9998$) within the range of 0.1-3.0 ng/ml for DDAC $r^2 \leq 0.9998$ within 0.51-12.3 ng/ml for C_{12} $r^2 \leq 1.0000$ within 1.26-15.1 ng/ml for C_{14} $r^2 \leq 0.9998$within 0.135-3.24 ng/ml for C_{16}
Specificity	No control chromatograms were provided. Chromatograms of standards contain shoulders which are consistent and do not appear to affect recovery. Peaks were well defined but occasionally asymmetrical. There appeared to be no carryover to the following chromatograms.

D. CONCLUSION

The LC/MS/MS Method GPL-MTH-056 is adequate for determining residues of DDAC and ADBAC (C_{12}, C_{14} and C_{16}) in solutions of 50% IPA, 10% ethanol, and corn oil, and from samples of dressing sponges and cotton percale. The validated method LOQs for the four analytes were 0.93-13.2 ng/mL from the 50% IPA solution, 4.9-70.5 ng/mL from the 10% ethanol solution, 1.05-14.0 ng/mL from the corn oil, 0.37-5.29 µg/sample from the dressing sponges, and 44-622 µg/sample from the sheets of percale.

E. REFERENCES

None.

EXECUTIVE SUMMARY:

MRID#46870703

A residue study was submitted examining the transfer of representative quaternary ammonium compounds (quats) from a treated laminate surface to representative food commodities. A laminate surface was chosen for the application as it is the most commonly used counter-top material in kitchens and other food preparation areas; and sliced bologna, apples and bread were chosen as the representative foods. The commercial test substance used for the study was Lemon Quat (Buckeye International, Inc.), which contains 2.54% of didecyl dimethyl ammonium chloride (DDAC) and 1.69% of n-alkyl dimethyl benzyl ammonium chloride (ADBAC; 40% C_{12}, 50% C_{14} and 10% C_{16}). DDAC is representative of Group I Quats and ADBAC is representative of Group II Quats.

The test was conducted in a simulated residential exposure room and utilized four replicate pieces of laminate (32 x 48 inches). An aqueous 1.5% dilution of the test substance was prepared and applied evenly to the laminate surfaces using a handle-held trigger sprayer. The diluted test substance was applied until run off. The treated laminate pieces were allowed to air dry for 2 hours following treatment, and the sections were not rinsed prior to exposure of the food. At 2-hours post-treatment, trimmed slices of bread (100 cm^2), apples (100 cm^2), and bologna (95 cm^2) were placed on the treated laminate for a 10-minute exposure duration. A single sample of each food was exposed on each of the 4 laminate replicates, for a total of 4 samples per food type. A separate 100 cm^2 area on each piece of laminate was used to determine the actual deposition of each compound. The selected area was wiped with two Excilon dressing sponges moistened with water followed by two dressing sponges moistened with 50% isopropanol (IPA) in water. The quantitative recovery of quat compounds from treated laminate by this wiping method was validated in a companion study. After exposure, treated samples of each type of food and the dressing sponges were stored in glass jars at ≤-10°C until analysis.

Samples of bologna, apple, bread and the dressing sponge wipes were analyzed for residues of each quat compound using adequate LC/MS/MS methods (Methods GPL-MTH-057 or -056), which were previously validated in separate studies. For both methods, residues are extracted with acetonitrile/water/formic acid, filtered, and diluted, and deuterated internal standards are then added for each analyte. Residues are analyzed by LC/MS/MS monitoring a single ion transition for each analyte. The validated limits of quantitation (LOQs) for the four analytes in the food samples are expressed in terms of µg compound per unit area, and are ~0.002-0.005 µg/cm^2 for bread, ~0.004-0.050 µg/cm^2 for apple, and ~0.004-0.053 µg/cm^2 for bologna. The validated LOQs for all four analytes in dressing sponges are ~0.37-5.3 µg/sample. The method was also validated in conjunction with the current study, using samples of each matrix field fortified at ~1x and 20x the LOQ or 10x and 200x the LOQ (bread). As the field-fortified samples were handled and stored under the same conditions as the treated sample, the fortified samples validated both the adequacy of the method and stability of residues in the various matrices during storage.

The total deposition of quat residues on the treated laminate surfaces were calculated using the residue data from the dressing sponge wipes with water and 50% IPA, and correcting the residues for the recovery of the wipe procedures. Total surface residues from all four laminate sections averaged 1.365 µg/cm^2 for DDAC, 0.537 µg/cm^2 for C_{12} ADBAC, 0.593 µg/cm^2 for C_{14} ADBAC, and 0.0906 µg/cm^2 for C_{16} ADBAC. For the wipe procedures, the majority of the residues for each compound were recovered in the initial water wipes, with the water wipes accounting for 89-95% of the recovered residues for DDAC, C_{12} ADBAC and C_{14} ADBAC, and 76.5% of the recovered residues for C_{16} ADBAC.

The transfer values (%) for each compound to food were calculated based on the amount ($\mu g/cm^2$) of each compound in each food sample (corrected for matrix recovery) and the amount of each compound originally on the treated surface (corrected for recovery from the wiping procedures). The transfer of all four quat compounds differed between the three representative foods, but was consistent among the four compounds for a given commodity. The average transference for DDAC and ADBAC (C_{12}, C_{14} and C_{16}) were 42.8-47.1% for bologna and 34.3-39.0% for apples, and the average transference for DDAC and ADBAC (C_{12} and C_{14}) were 0.9-1.0% for bread. The transference of C_{16} ADBAC could not be calculated for bread as residues of this compound were <LOQ in the bread samples. Over a 10-minute exposure period to treated laminate surfaces, the average transfer of residues from all four compounds was 44.3% for bologna, 37.4% for apples, and 0.89% for bread.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the residue data are classified as scientifically acceptable for the purpose for which it was intended.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an adverse impact on the validity of the study.

A. BACKGROUND INFORMATION

DDAC and ADBAC are antimicrobials used in several types of applications, such as indoor and outdoor hard surfaces (e.g. walls, floors, tables, toilets and fixtures) eating utensils, laundry, carpets, agricultural tools and vehicles, egg shells, shoes, milking equipment and udders, humidifiers, medical instruments, human remains, ultrasonic tanks, reverse osmosis units and water storage tanks. There are also DDAC and ADBAC-containing products that are used in residential and commercial swimming pools, in aquatic areas such as decorative ponds and fountains, and in industrial process and water systems such as re-circulating cooling water systems, drilling muds and packer fluids, oil well injection and wastewater system. Additionally, DDAC and ADBAC-containing products are used for wood preservation.

The chemical structure and nomenclature of DDAC and ADBAC, and the physicochemical properties of the technical grade of DDAC and ADBAC are presented in Tables A.1 and A.2.

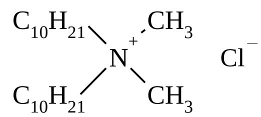
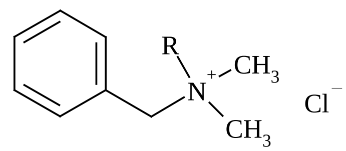
TABLE A.1. DDAC and ADBAC Nomenclature.	
Compound	
Common name	DDAC
Company experimental name	DDAC
IUPAC name	Didecyl dimethyl ammonium chloride
CAS name	Didecyl dimethyl ammonium chloride
CAS Registry number	7173-51-5
Compound	 R= C12, C14, C16
Common name	ADBAC (40% C_{12}, 50% C_{14}, and 10% C_{16})
IUPAC Name	n-Alkyl dimethyl benzyl ammonium chloride
CAS Name	n-Alkyl dimethyl benzyl ammonium chloride
CAS Registry number	68424-85-1

TABLE A.2. Physicochemical Properties of DDAC and ADBAC			
Parameter	Value (DDAC)	Value (ADBAC) [1]	Reference
Molecular weight	326.08	377.83	DDAC and ADBAC REDs (2006)
Melting point	228.81°C	241.02°C	
Density (at 25°C)	0.9216 g/cm^3	0.9429 g/cm^3	
Water solubility	Completely Soluble	Soluble	
Solvent solubility	Not stated	Soluble in Alcohols	
Vapor pressure (at 25°C)	2.33 x 10^{-11} mm Hg	3.53 x 10^{-12} mm Hg	

B. EXPERIMENTAL DESIGN

The study was designed to 1) measure the transferability of dried residues of representative quaternary ammonium chloride compounds (DDAC, C_{12} ADBAC, C_{14} ADBAC and C_{16} ADBAC) from treated laminate surfaces to representative food commodities, and 2) determine the degree of similarity of residue transfer among the representative quaternary ammonium compounds. The resulting data could then be used to refine estimates of indirect dietary exposure from quaternary ammonium compounds.

A laminate surface was chosen for the application as it is the most commonly used counter-top material in kitchens and other food preparation areas. Three representative foods were selected for the study: sliced apples (Red Delicious), bologna (Oscar Myer Beef Bologna), and white bread (Wonder Classic Wonder Bread). Apples were selected as they have been previously used as representative fruit in other studies of food transfer and are a common ingredient in children's diets. In addition, the relatively high water content of apples, makes them a good surrogate for both fruits and vegetables. Bologna was selected as it is a common snack and sandwich ingredient, and most pesticides tested for transfer from surfaces typically transferred equally or more efficiently to meat than cheese, making bologna a reasonable surrogate for cheese. Bread was selected as it is a common ingredient for all sandwiches, tends to be the most likely component to contact a countertop, and is one of the most commonly consumed foods. Bread has less water content than meat, cheese, vegetables or fruit, and thus seemed appropriate for measurement in this study as a transfer matrix. Bread is also similar to other dry food e.g. almonds, also categorized as "pieces."

B.1. Study Methods

The test substance used for this study is Lemon Quat (Table B.1.1), which is a commercially available formulation containing a mixture of DDAC and ADBAC (40% C_{12}, 50% C_{14} and 10% C_{16}). The formulation was diluted with water to yield a 1.5% dilution for application, and the concentration of the various quaternary ammonium compounds in the diluted spray was 0.0025-0.038%. The application and subsequent exposure of food samples was conducted in a simulated residential exposure room at the test facility.

The diluted test substance was applied to four replicate 32x48 inch pieces of laminate using a hand-held trigger sprayer (Table B.1.2). The laminate pieces were oriented horizontally for application, and the spray was applied evenly as possible from top to bottom and side to side until each section was wet enough for run off. The four laminate pieces were allowed to air dry for 2 hours following treatment, and the sections were not rinsed prior to exposure of the food. The 2-hour drying time was selected as both DDAC and ADBAC are stable on the surface for this duration, and the time more than satisfies the label-required contact time for surface disinfection.

Pieces of bread had the crust trimmed with a knife to the dimensions of 10 x 10 cm. Apples were cut by hand into transverse sections 1/8th inch thick starting at the blossom end; rectangular pieces were then cut from the rounds and fitted into a 10 x 10 cm outline. Bologna slices were used without modification from the refrigerated package and had diameter of 11 cm, for a surface area of 95 cm^2.

At two hours after application, a "sheen" of liquid was still noted on some areas ($\leq$300 cm^2) of each sheet of treated laminate. These areas were not used for placement of food or for sampling surface residues. A single sample of each food item was randomly placed on a dried area of each piece of laminate, and the food samples were left in contact with the treated surface for 10 minutes. The 10-min duration for exposure was selected as it was considered a reasonable time required for preparation of food items and correlates with exposure durations previously used in studies by EPA/ORD.

To determine the actual amounts of the test substances applied, a randomly selected 100 cm^2 area of each laminate section was designated for wiping with Excilon dressing sponges. The selected areas excluded areas where food samples were placed. A stainless steel template with a 10 x 10 cm cutout was placed over the area, and the area was first wiped with two dressing sponges pre-moistened with 5 ml water followed by wiping with two additional sponges each saturated with 5 ml of 50% IPA solution. The quantitative recovery of applied compounds by this wiping method was validated in a separate study (DER 46870704). The water and 50% IPA wipe sponges were then placed into separate jars for analysis.

TABLE B.1.1. Test Substance.

Representative Quat Compounds	End-use product	Concentration of a.i.	Application Solution	Final a.i. concentration (%)
DDAC ADBAC (C_{12}, C_{14} and C_{16})	Lemon Quat (Buckeye International, Inc.)	2.54% DDAC and 1.69%ADBAC (40% C_{12}, 50% C_{14} and 10% C_{16})	1.5% Aqueous dilution	DDAC 0.038% C_{12} ADBAC 0.010% C_{14} ADBAC 0.013% C_{16} ADBAC 0.0025%

TABLE B.1.2. Study Site and Use Pattern.

Test site	Treated surface	Application Solution	Application information				
			Method	Rate	No. of Appls.	Drying Time	Rinse
Simulated residential exposure room (72 ± 4°F; 45 ± 20% RH; and 0.6 ± 0.1 air changes/hour)	32” x 48” laminate surface (WisonArt® Basic type #107	1.5% aqueous dilution of Lemon Quat	Trigger Sprayer to horizontal surface	To Run Off	1	2 hours	None

B.2. Sample Handling and Preparation

After 10 minutes of exposure to the treated laminate surface, the food samples were collected and placed into glass jars. The water and 50% IPA wipes were also placed into glass jars, and all samples were stored at ≤-10°C until extraction and analysis.

B.3. Analytical Methodology

Samples of the dressing sponges, bologna, bread and apple slices were analyzed for residues of DDAC and ADBAC (C_{12}, C_{14}, and C_{16}) using two related LC/MS/MS method (Methods GPL-MTH-057 and GPL-MTH-056). These methods are identical in regards to extraction and analysis, and differ only in the matrices analyzed. Prior to initiating the study, these methods were validated for the recovery of DDAC and ADBAC (C_{12}, C_{14}, and C_{16}) from the three representative foods and dressing sponges. Detailed descriptions of the methods and the results from the method validations are reported in DERs 46870701 and 46870702.

For both methods, residues of DDAC and ADBAC are extracted with acetonitrile/water/formic acid (70:30:0.016, v:v) by either homogenization for slices of bread, apples and bologna or by shaking for dressing sponges. Aliquots of the extracts are filtered and diluted, and deuterated internal standards are added for each analyte. Residues are then analyzed by LC/MS/MS using a C_{18} column and a mobile phase gradient of water to acetonitrile, each containing 0.2% formic acid. A single ion transition is monitored for each analyte, and internal standards are used for quantitation of each compound. The validated LOQs for the four analytes are expressed in terms of µg compound per unit area for the food commodities and in terms of µg/sample for the dressing sponges. The validated LOQ for each analyte are reported in Table B.3.

In addition, the methods were validated concurrently with this study. Duplicate control samples of dressing sponges, bologna, and apple slices were fortified with each of the representative analytes at ~1x and 20x the LOQ, and duplicate control samples of bread were fortified with each analyte at 10x and 200x the LOQ. The fortified samples were spiked during the 2-hour drying period for the treated laminate, and then placed in frozen storage under the same conditions as the treated samples. As such, the recoveries from the fortified samples validated both the adequacy of the method and stability of residues in the various matrices during storage.

TABLE B.3. Summary of LOQs for Bread, Apple, Bologna and Dressing Sponges[1].

Matrix	Analyte LOQ [2]			
	C_{12} ADBAC	C_{14} ADBAC	C_{16} ADBAC	DDAC
Bread	0.00163 µg/cm^2	0.00188 µg/cm^2	0.00360 µg/cm^2	0.00503 µg/cm^2
Apple	0.0163 µg/cm^2	0.0188 µg/cm^2	0.00360 µg/cm^2	0.0503 µg/cm^2
Bologna	0.0172 µg/cm^2	0.0198 µg/cm^2	0.00379 µg/cm^2	0.0529 µg/cm^2
Dressing Sponges	1.70 µg/sample	1.95 µg/sample	0.370 µg/sample	5.29 µg/sample

1 LOQs are summarized from MRIDs 46870701 and 46870702, in which the analytic method was validated.

C. RESULTS AND DISCUSSION

The LC/MS/MS methods used to analyze samples of dressing sponges, bologna, bread and apples for residues of the representative quats were adequately validated in conjunction with the analysis of treated samples. The recoveries of each analyte from fortified samples are presented in Table C.1. Acceptable recoveries were obtained for each analyte in each matrix. For all four matrices, the average recoveries (with standard deviations) were 74.8-92.8% (1-4%) for DDAC, 81.4-96.5% (2-4%) for C_{12} ADBAC, 78.4-98.3% (1-7%) for C_{14} ADBAC, and 61.6-88.6% (3-6%) for C_{16} ADBAC. Although recoveries for C_{16} ADBAC were below 70% from several apple and bread samples, the variability of the recoveries from these matrices was also low (5-6% CV); therefore, the method was deemed adequate.

Samples were stored frozen at ≤-10°C, except for when thawed for analysis (Table C.2). Because the fortified control samples were spiked just prior to placing the other food samples on the treated laminate, and the fortified samples were stored under the same conditions as the treated samples, the method validation samples also account for the stability of the residues under the storage conditions for the duration of the study.

The total deposition of quat residues on the treated laminate surfaces were calculated using the data from the dressing sponge wipes with water and 50% IPA (Table C.3.1). For the four treated laminate sections, total average residues from both the water and 50% IPA wipes were 1.14 µg/cm^2 for DDAC, 0.4107 µg/cm^2 for C_{12} ADBAC, 0.4539 µg/cm^2 for C_{14} ADBAC, and 0.0693 µg/cm^2 for C_{16} ADBAC. The levels of the various quats reflected their relative concentrations in the test substance. For all four quats the majority of the residues were recovered in the initial water wipes, with the water wipes accounting for 89-95% of the recovered residues for DDAC, C_{12} ADBAC and C_{14} ADBAC, and 76.5% of the recovered residues for C_{16} ADBAC. To calculate the actual levels of each quat deposited on the laminate, the study authors corrected the recovered residues using factors of 0.835 for DDAC and 0.765 for ADBAC (C_{12}, C_{14} and C_{16}) in order to account for the recovery of the quats from the surface. These recovery values were determined in a companion study (MRID 46870704) in which laminate surfaces were treated with known amounts of either DDAC or C_{14} ADBAC, and then wiped with water and 50% IPA saturated dressing sponges. When the recovered residues from the current study are corrected for recovery by the wiping procedures, the total surface residues were 0.909-1.805 µg/cm^2 for DDAC, 0.340-0.707 µg/cm^2 for C_{12} ADBAC, 0.413-0.805 µg/cm^2 for C_{14} ADBAC, and 0.0599-0.1224 µg/cm^2 for C_{16} ADBAC. The average (±S.D) of total surface residues from all four laminate sections were 1.365± 0.374 µg/cm^2 for DDAC, 0.537 ± 0.164 µg/cm^2 for C_{12} ADBAC, 0.593 ± 0.162 µg/cm^2 for C_{14} ADBAC, and 0.0906 ± 0.026 µg/cm^2 for C_{16} ADBAC. The average values for total surface residues were used as the basis for calculating transference from the various food commodities.

Residues of each quat compound recovered from the four replicates of each food commodity are presented in Table C.3.2. In order to more accurately determine the percent transfer of residues to the various food commodities, the recovered residues from the three foods were corrected using the average recovery of each analyte from the fortified samples of each food (Table C.1). Corrected residues of DDAC were 0.405-0.758 µg/cm^2 for bologna, 0.267-0.913 µg/cm^2 for apples, and 0.0076-0.0194 µg/cm^2 for bread. Corrected residues of C_{12} ADBAC were 0.165-0.311 µg/cm^2 for bologna, 0.096-0.366 µg/cm^2 for apples, and 0.0033-0.0073 µg/cm^2 for bread. Corrected residues of C_{14} ADBAC were 0.148-0.317 µg/cm^2 for bologna, 0.096-0.370 µg/cm^2 for apples, and 0.0032-0.0078 µg/cm^2 for bread. Corrected residues of C_{16} ADBAC were 0.0226-0.0514 µg/cm^2 for bologna, 0.0153-0.0307 µg/cm^2 for apples, and <LOQ for bread.

Residue transfer values (%) for each quat were calculated for the individual food samples by dividing the corrected residues in each food sample by average total surface residues on all four laminate sections for each quat compound. The individual and average transfer values for each quat in each food commodity are reported in Tables C.3.2 and C.4.

For a given food type, the surface transfer of residues was similar among all four representative quat compounds. For bologna, the average transference for DDAC, C_{12}, C_{14} and C_{16} ADBAC were 43.0%, 47.1%, 42.8% and 44.2%, respectively. For apple slices, the average transference for DDAC, C_{12}, C_{14} and C_{16} ADBAC were 39.0%, 38.5%, 34.3%, and 37.6%, respectively. For bread, the average transference for DDAC, C_{12}, and C_{14} ADBAC were 1.0%, 0.9%, 0.9%, respectively; the transference of C_{16} ADBAC could not be calculated for bread as residues of this compound were <LOQ in the bread samples.

Over a 10-minute exposure period to treated laminate surfaces, the average transfer (±S.D.) of residues from all four quat compounds was 44.3 ± 11.6% for bologna, 37.4 ± 19.3% for apple slices, and 0.89 ± 0.3% for bread.

TABLE C.1. Summary of Concurrent Recoveries of DDAC and ADBAC from Dressing Sponges, Bologna, Apples and Bread. [1]					
Analyte	Matrix	Spike Level [2]	Sample Size (n)	Recoveries (%)	Mean ± Std. Dev. (%)
DDAC	Dressing Sponges	5.03 µg/sample	2	92.6, 93.8	92.8 ± 1.0
		101 µg/sample	2	91.5, 93.3	
	Bologna	0.0259 µg/cm^2	2	86.6, 85.8	85.9 ± 1.0
		1.06 µg/cm^2	2	84.5, 86.7	
	Apples	0.0503 µg/cm^2	2	71.6, 73.2	74.9 ± 3.5
		1.01 µg/cm^2	2	79.8, 75.1	
	Bread	0.0503 µg/cm^2	2	94.0, 91.5	89.8 ± 3.8
		1.01 µg/cm^2	2	85.4, 88.2	
C_{12} ADBAC	Dressing Sponges	1.63 µg/sample	2	96.3, 92.0	93.4 ± 2.3
		32.6 µg/sample	2	91.1, 94.2	
	Bologna	0.0172 µg/cm^2	2	94.2, 91.9	96.5 ± 4.4
		0.343 µg/cm^2	2	98.0, 102	
	Apples	0.0163 µg/cm^2	2	80.4, 78.5	81.4 ± 2.4
		0.326µg/cm^2	2	83.4, 83.4	
	Bread	0.0.0163 µg/cm^2	2	95.1, 89.0	92.5 ±2.6
		0.326 µg/cm^2	2	92.3, 93.6	
C_{14} ADBAC	Dressing Sponges	1.88 µg/sample	2	92.6, 97.9	94.8 ± 2.7
		37.5 µg/sample	2	96.0, 92.5	
	Bologna	0.0198 µg/cm^2	2	94.4, 90.9	98.3 ± 7.1
		0.385 µg/cm^2	2	101, 107	
	Apples	0.0188 µg/cm^2	2	76.6, 77.1	78.4 ±2.0
		0.375 µg/cm^2	2	81.1, 78.7	
	Bread	0.0188 µg/cm^2	2	86.2, 86.7	87.1 ±0.8
		0.375 µg/cm^2	2	87.5, 88.0	
C_{16} ADBAC	Dressing Sponges	0.360 µg/sample	2	88.6, 90.3	87.4 ±2.7
		7.19 µg/sample	2	84.1, 86.5	
	Bologna	0.004 µg/cm^2	2	85.5, 84.7	88.6 ± 5.8
		0.076 µg/cm^2	2	86.8, 97.2	
	Apples	0.004 µg/cm^2	2	69.7, 67.5	71.7 ± 4.5
		0.072 µg/cm ^{2}	2	78.0, 71.6	
	Bread	0.004 µg/cm^2	2	64.4, 63.9	61.6 ± 3.0
		0.072 µg/cm^2	2	59.5, 58.6	

1 Samples were field fortified at the same time as treated samples were collected.

1 Samples of dressing sponges, bologna and apples were field fortified at levels equivalent to 1x and 20x the LOQ, and bread samples were fortified at 10x and 200x the LOQ.

TABLE C.2. Summary of Storage Conditions.			
Matrix	Storage Temperature (°C)	Actual Storage Duration (days)	Interval of Demonstrated Storage Stability (days)
Bologna	<-10	<13 [1]	13 [2]
Apple			
Bread			

1 Sample analyses were completed within 13 days of study initiation.
2 As fortified control samples were spiked prior to exposure of food to the treated surfaces and stored under the same conditions as the treated samples, the recovery data (Table C.2) for these samples support the stability of residues.

Table C.3.1. Residues of DDAC and ADBAC (C_{12}, C_{14}, and C_{16}) Recovered from a Treated Laminate Surface Using Dressing Sponges Moistened with Water and 50% Isopropanol.

Analyte	Measured Residues from Wipes (μg/cm²)			% Total Recovered [1]		Total Surface Residues [1] (μg/cm²)
	water	50% IPA	Total	water	50% IPA	
DDAC	1.35	0.157	1.51	89.6	10.4	1.805
	0.655	0.104	0.759	86.3	13.7	0.909
	0.953	0.104	1.06	90.2	9.8	1.266
	1.08	0.135	1.22	88.9	11.1	1.455
average	**1.01**	**0.125**	**1.14**	**89.0**	**11.0**	**1.365**
C_{12} ADBAC	0.516	0.025	0.541	95.4	4.6	0.707
	0.238	0.0219	0.2599	91.6	8.4	0.340
	0.344	0.0167	0.3607	95.4	4.6	0.472
	0.462	0.0191	0.4811	96.0	4.0	0.629
average	**0.39**	**0.0207**	**0.4107**	**95.0**	**5.0**	**0.537**
C_{14} ADBAC	0.557	0.0586	0.6156	90.5	9.5	0.805
	0.275	0.0408	0.3158	87.1	12.9	0.413
	0.39	0.0345	0.4245	91.9	8.1	0.555
	0.414	0.0457	0.4597	90.1	9.9	0.601
average	**0.409**	**0.0449**	**0.4539**	**90.1**	**9.9**	**0.593**
C_{16} ADBAC	0.0714	0.0222	0.0936	76.3	23.7	0.1224
	0.033	0.0128	0.0458	72.1	27.9	0.0599
	0.0548	0.0144	0.0692	79.2	20.8	0.0905
	0.0528	0.0156	0.0684	77.2	22.8	0.0894
average	**0.053**	**0.0163**	**0.0693**	**76.5**	**23.5**	**0.0906**

1 Percent of total recovered by water and 50% IPA wipes with dressing sponges.
2 Total surface residues were calculated using data from another study (MRID 46870704) which indicated that water and 50% IPA wipes of treated surfaces quantitatively recovered 83.5% of applied DDAC and 76.5% of applied C_{14} ADBAC. The recovery value for C_{14} ADBAC was used for C_{12} and C_{16} ADBAC.

TABLE C.3.2. Residues of DDAC and ADBAC (C_{12}, C_{14}, and C_{16}) Transferred to Representative Food Commodities from a Treated Laminate Surface. [1]

Food Matrix	Analyte	Average Total Surface Residues (μg/cm²) [2]	Measured Food Residues (μg/cm²)	Corrected Food Residues (μg/cm²) [3]	% Transfer [4]
Bologna	DDAC	1.365	0.651, 0.465, 0.348, 0.561	0.758, 0.541, 0.405, 0.653	55.3, 39.5, 29.6, 47.7
	C_{12} ADBAC	0.537	0.276, 0.241, 0.159, 0.300	0.286, 0.250, 0.165, 0.311	53.3, 46.6, 30.7, 57.9
	C_{14} ADBAC	0.593	0.297, 0.243, 0.145, 0.312	0.302, 0.247, 0.148, 0.317	50.9, 41.7, 25.0, 53.5
	C_{16} ADBAC	0.0906	0.0449, 0.0315, 0.0200, 0.0455	0.0507, 0.0356, 0.0226, 0.0514	56.0, 39.3, 24.9, 56.7
Apples	DDAC	1.365	0.684, 0.389, 0.328, 0.200	0.913, 0.519, 0.438, 0.267	66.6, 37.9, 32.0, 19.5

TABLE C.3.2. Residues of DDAC and ADBAC (C_{12}, C_{14}, and C_{16}) Transferred to Representative Food Commodities from a Treated Laminate Surface. [1]

Food Matrix	Analyte	Average Total Surface Residues (µg/cm²) [2]	Measured Food Residues (µg/cm²)	Corrected Food Residues (µg/cm²) [3]	% Transfer [4]
	C_{12} ADBAC	0.537	0.298, 0.163, 0.135, 0.078	0.366, 0.200, 0.166, 0.096	68.2, 37.2, 30.9, 17.8
	C_{14} ADBAC	0.593	0.290, 0.149, 0.123, 0.075	0.370, 0.190, 0.157, 0.096	62.4, 32.0, 26.5, 16.2
	C_{16} ADBAC	0.0906	0.0472, 0.0220, 0.0176, 0.0110	0.0658, 0.0307, 0.0245, 0.0153	72.6, 33.9, 27.0, 16.9
Bread	DDAC	1.365	0.0131, 0.0068, 0.0095, 0.0174	0.0146, 0.0076, 0.0106, 0.0194	1.07, 0.55, 0.77, 1.42
	C_{12} ADBAC	0.537	0.0046, 0.0027, 0.0031, 0.0067	0.0050, 0.0029, 0.0033, 0.0073	0.96, 0.54, 0.62, 1.35
	C_{14} ADBAC	0.593	0.0044, 0.0028, 0.0035, 0.0068	0.0051, 0.0032, 0.0041, 0.0078	0.86, 0.54, 0.69, 1.31
	C_{16} ADBAC	0.0906	<LOQ	<LOQ	NC

1 Food samples were left in contact with the dried, treated surface for 10 minutes.
2 Data from Table C.3.1.
3 Corrected for corresponding average field fortification recovery from Table C.1.
4 %Transfer = (corrected residues in food/average surface residues) x 100.
NC = not calculated as C_{16} ADBAC were <LOQ in/on bread.

Table C.3.3. Summary of ADBAC and DDAC Residues and % Transference.

Food Commodity	Analyte	Average Total Surface Residues [1] (µg/cm²)	% Transfer [2]					
			n	Min.	Max.	Median	Mean	Std. Dev.
Bologna	DDAC	1.365	4	29.6	55.3	43.6	43.0	11.0
	C_{12} ADBAC	0.537	4	30.7	57.9	50.0	47.1	11.9
	C_{14} ADBAC	0.593	4	25.0	53.5	46.3	42.8	12.9
	C_{16} ADBAC	0.0906	4	24.9	56.7	47.7	44.2	15.2
	Average Quat Transference		**16**	**24.9**	**57.9**	**47.12**	**44.3**	**11.6**
Apple	DDAC	1.365	4	19.5	66.6	35.0	39.0	19.9
	C_{12} ADBAC	0.537	4	17.8	68.2	34.1	38.5	21.4
	C_{14} ADBAC	0.593	4	16.2	62.4	29.3	34.3	19.9
	C_{16} ADBAC	0.0906	4	16.9	72.6	30.5	37.6	24.4
	Average Quat Transference		**16**	**16.2**	**72.6**	**37.34**	**37.4**	**19.3**
Bread	DDAC	1.365	4	0.6	1.4	0.9	1.0	0.4
	C_{12} ADBAC	0.537	4	0.5	1.4	0.8	0.9	0.4
	C_{14} ADBAC	0.593	4	0.5	1.3	0.8	0.9	0.3
	C_{16} ADBAC	0.0906	4	<LOQ	<LOQ	NA	NA	NA
	Average Quat Transference		**12** [3]	**0.5**	**1.4**	**0.815**	**0.89**	**0.3**

1 Data from Table C.3.1.
2 %Transfer = (corrected residues in food/average surface residues) x 100.
3 As residues of C_{16} ADBAC were <LOQ in/on bread, the % transfer of C_{16} ADBAC was not calculated or included in calculating the average quat transference.
NA = not applicable.

D. CONCLUSION

The study is adequate for the purposes for which it was intended. The study quantitatively determined the transference (%) of representative quad compounds from treated laminate surfaces to slices of bologna, apples and bread during a 10-mintue exposure period. The transfer values were calculated based on the amount (µg/cm^2) of each quat in each food, (corrected for matrix recovery) and the amount of each compound originally on the treated surface (corrected for recovery from the wiping procedures).

The transfer of all four compounds (DDAC, C_{12} ADBAC, C_{14} ADBAC and C_{16} ADBAC) differed between the three representative foods, but was consistent among the four compounds for a given commodity. The overall transfer of all four compounds over the 10-minute exposure averaged 44.3% for bologna, 37.4% for apples, and 0.89% for bread. These data indicate that the transfer of quat residues is more dependent on the food type than the specific type of quat compound. Therefore, for purposes of risk assessment, these data can be used to estimate transfer of surface residues from all types of quats to the tested food commodities.

E. REFERENCES

None

EXECUTIVE SUMMARY:

MRID# 46870704

A residue study was submitted examining the deposition of two representative quaternary ammonium compounds (quats) onto a laminate surface using different application regimes. The representative quat compounds selected for the test were didecyl dimethyl ammonium chloride (DDAC; Group I Quat) and C_{14}-alkyl dimethyl benzyl ammonium chloride (C_{14} ADBAC, Group II Quat). The study also examined the recovery of dried residues of DDAC and C_{14} ADBAC from laminate surfaces using dressing sponges moistened with water and 50% isopropanol in water (50% IPA).

The study was conducted in a simulated residential exposure room and utilized four replicate pieces of laminate (81 cm x 122 cm or 61 cm x 81 cm) for each type of application. Based on labeled use directions for end-use products, the following four application methods were tested: a wipe-on application using a commercially available presaturated wipe (Albertsons Disinfecting Wipes); a wipe-on application using a rag saturated with a 1.5% diluted commercial solution (Lemon Quat); a spray application of the same dilute solution using a hand-held trigger sprayer; and a spray application using a commercially available aerosol spray (Clorox Disinfecting Spray). Three different end-use products containing DDAC and C_{14} ADBAC were utilized for these tests, and the final concentrations of these compounds in the application solutions were 0.0199-0.0945% for DDAC and 0.0125-0.126% for C_{14} ADBAC. For the wipe-on applications, the treatment solution was applied by wiping each laminate piece from top to bottom and from side to side with overlapping strokes. For the two spray applications, the treatment solutions were sprayed onto the laminate as evenly as possible from top to bottom and side to side until each section was wet enough for run off. Following each type of application, the laminate pieces were allowed to air dry of 2 hours prior to testing for deposition of DDAC and C_{14} ADBAC.

Following drying, a single, randomly selected, 10 cm x10 cm section on each laminate piece was wiped to determine the deposition of DDAC and C_{14} ADBAC. Each section was initially wiped with an Excilon dressing sponge moistened with water followed by two dressing sponges moistened with 50% IPA. The water and 50% IPA dressing sponges were then placed in separate glass jars and stored at ≤-10°C until analysis (<7 days). The quantitative recovery of dried DDAC and C_{14} ABDAC residues using this wiping method was validated in a preliminary test; the average total recovery by wiping with water and 50% IPA using dressing sponges was determined to be 83.5% for DDAC and 76.5% for C_{14} ADBAC.

Residues of DDAC and C_{14} ADBAC in/on the dressing sponges were analyzed using an adequate LC/MS/MS method (Methods GPL-MTH-056), which was previously validated in a separate study (MRID# 46870702). For this method, residues are extracted with acetonitrile/water/formic acid, filtered, and diluted. Deuterated internal standards for each analyte are then added to the extracts. Residues are analyzed by LC/MS/MS, monitoring a single ion transition for each analyte. The validated limit of quantitation (LOQ) for residues in the dressing sponges is 5.29 µg/sample for DDAC and 1.95 µg/sample for C_{14} ADBAC. The method was also validated in conjunction with the analysis of study samples, using control samples of dressing sponges fortified separately with each analyte at ~1x and 20x the LOQ.

The majority of the recovered residues for both compounds were recovered in the initial water wipe for each type of application. For the wipe-on type applications, the percent of recovered residues in the water saturated dressing sponges averaged 51-53% for C_{14} ADBAC and 55-65% for DDAC. For the two sprayer type applications, the percent of recovered residues in the water saturated dressing sponges averaged 68-71% for C_{14} ADBAC and 73-78% for DDAC. If the initial water wipe is assumed to approximate a surface rinse with water, and the recovery for the water wipes is adjusted to account for the overall recovery of both DDAC and C_{14} ADBAC by the surface wipe method, then a water rinse should remove approximately 52% of ADBAC and 60% of DDAC residues deposited from a wipe-on type application, and 70% of ADBAC and 76% of DDAC residues deposited by a sprayer type application..

The total surface residues of both compounds were determined by correcting the recovered residues to account for the average recovery of DDAC (83.5%) and ADBAC (76.5%) using the dressing sponge wiping procedures. Total average surface residues of DDAC and C_{14} ADBAC were respectively 0.0181 and 0.0297 µg/cm^2 for the presaturated wipe application, 0.0769 and 0.0271 µg/cm^2 for the rag wipe application of the dilute solution, 1.30 and 0.536 µg/cm^2 for the trigger spray application of the dilute solution, and 4.10 and 5.24 µg/cm^2 for aerosol spray application. Although concentrations of the two compounds varied in the application solutions, the above data indicate that the wipe-on applications deposited substantially lower amounts of the representative quat compounds than the spray applications. For the two applications which used the same dilute solution of Lemon Quat, the levels of DDAC and C_{14} ADBAC were respectively 17x and 20x higher for the trigger sprayer application than for the rag wipe application.

Both the trigger sprayer and aerosol spray applications had similar levels of deposition of both DDAC and C_{14} ADBAC (~3500-4300 µg/cm^2), and the amounts deposited by the spray applications were approximately 20x greater than the amounts of both compounds deposited by the rag wipe application (~200 µg/cm^2) and 40x more than the amounts deposited by the presaturated wipe application (~100 µg/cm^2). The data indicate that at the same concentration, the deposition of difference quat compounds will be similar for a given application method, and that sprayer type applications will result in substantially higher levels of surface residues than wipe-on applications.

<u>STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS</u>:

Although this was essentially a non-guideline study, there were no major deficiencies noted in the study, and the study is adequate for the purposes for which it was intended.

<u>COMPLIANCE</u>:

Signed and dated Good Laboratory Practices (GLP), Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

DDAC and ADBAC are antimicrobials used in several types of applications, such as indoor and outdoor hard surfaces (e.g. walls, floors, tables, toilets, and fixtures) eating utensils, laundry, carpets, agricultural tools and vehicles, egg shells, shoes, milking equipment and udders, humidifiers, medical instruments, human remains, ultrasonic tanks, reverse osmosis units, and water storage tanks. There are also DDAC and ADBAC-containing products that are used in residential and commercial swimming pools, in aquatic areas such as decorative ponds and fountains, and in industrial process and water systems such as re-circulating cooling water systems, drilling muds and packer fluids, oil well injection, and wastewater systems. Additionally, DDAC and ADBAC-containing products are used for wood preservation.

The chemical structure and nomenclature of DDAC and ADBAC, and the physicochemical properties of the technical grade of DDAC and ADBAC are presented in Tables A.1 and A.2.

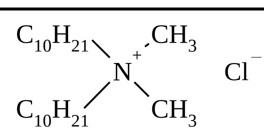
TABLE A.1. DDAC and ADBAC Nomenclature.	
Compound	
Common name	DDAC
Company experimental name	DDAC
IUPAC name	Didecyl dimethyl ammonium chloride

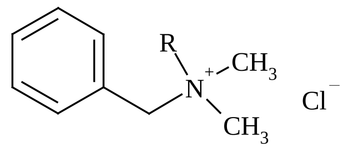
TABLE A.1. DDAC and ADBAC Nomenclature.	
CAS name	Didecyl dimethyl ammonium chloride
CAS Registry number	7173-51-5
Compound	 R= C12, C14, C16
Common name	ADBAC (40% C_{12}, 50% C_{14}, and 10% C_{16})
IUPAC Name	n-Alkyl dimethyl benzyl ammonium chloride
CAS Name	n-Alkyl dimethyl benzyl ammonium chloride
CAS Registry number	68424-85-1

TABLE A.2. Physicochemical Properties of DDAC and ADBAC			
Parameter	Value (DDAC)	Value (ADBAC) [1]	Reference
Molecular weight	326.08	377.83	DDAC and ADBAC REDs (2006)
Melting point	228.81°C	241.02°C	
Density (at 25°C)	0.9216 g/cm^3	0.9429 g/cm^3	
Water solubility	Completely Soluble	Soluble	
Solvent solubility	Not stated	Soluble in Alcohols	
Vapor pressure (at 25°C)	2.33 x 10^{-11} mm Hg	3.53 x 10^{-12} mm Hg	

B. EXPERIMENTAL DESIGN

The study was designed to measure the deposition of residues of two representative quaternary ammonium chloride compounds (DDAC and C_{14} ADBAC) on laminate surfaces following treatment with commercial formulations containing quats using four typical application methods. The quats selected for analysis are representative of Group I Quats (DDAC) and Group II Quats (C_{14} ADBAC). A secondary objective of the study was to determine the quantitative recovery of these compounds from laminate surfaces using wipes with dressing sponges saturated with water and 50% IPA.

A laminate surface was used for the applications as it is the most commonly used counter-top material in kitchens and other food preparation areas. The typical application methods tested included use of a pre-saturated wipe; a rag wipe with a dilute solution; trigger spray with a dilute solution; and an aerosol spray.

B.1. Study Methods

Prior to initiating the main study, a test was conducted to determine the quantitative recovery of DDAC and C_{14} ADBAC from fortified laminate pieces using dressing sponges saturated with water and 50% IPA. For this test, four replicate pieces of laminate (10 cm x 10 cm) were each fortified with 100 µL of Lemon Quat dissolved in ethanol (1.2669 g/50mL); 24.4 µg of C_{14} ADBAC and 65.6 µg of DDAC. The pieces were allowed to air dry for 2 hours. Each section was then wiped horizontally and vertically, first with a dressing sponge moistened with water (5 mL) and then with two dressing sponges moistened with 50% IPA in water (5 mL/sponge). The levels of DDAC and C_{14} ADBAC were then determined in the water and 50% IPA wipes.

For the main study, four different application methods were compared using three different end-use products (Table B.1.1). The application methods were based on the labeled use direction for each product. Four replicate pieces of laminate were used for each application method and the study was conducted in a simulated residential exposure room at the test facility (Table B.1.2).

TABLE B.1.1. Test Substances.

End-use products	Manufacturer	EPA Reg. No. (Lot No.)	Formulation type	Active ingredients [1]	% a.i. [2]
Lemon Quat	Buckeye International, Inc	47371-131-559 (5076-E5K)	Liquid concentrate	DDAC C_{12} ADBAC C_{14} ADBAC C_{16} ADBAC	2.54 0.676 0.845 0.169
Albertsons Disinfecting Wipes	Albertson's Inc.	47371-36-67619 (85118-458)	Presaturated wet wipes	DDAC C_{12} ADBAC C_{14} ADBAC C_{16} ADBAC ODAC DODAC	0.01995 0.0213 0.0266 0.0053 0.0399 0.01995
Clorox Disinfecting Spray	Clorox Professional Products Co.	67619-3 (Y45050-1)	Aerosol spray	DDAC C_{12} ADBAC C_{14} ADBAC C_{16} ADBAC ODAC DODAC Ethanol	0.0945 0.101 0.126 0.025 0.189 0.0945 65

1 DDAC- Didecyl dimethyl ammonium chloride; ADBAC - n-alkyl dimethyl benzyl ammonium chloride; ODAC - octyl decyl ammonium chloride; and DODAC - dioctyl dimethyl ammonium chloride.

2 Concentrations for the various ADBAC compounds are based on the following ratio: 40% C_{12}, 50% C_{14} and 10% C_{16}.

TABLE B.1.2. Study Site and Use Pattern.

Test site	Treated surface	Application Information					
		Type	A.I. [1] (%)	Method	No. of Appls.	Drying Time	Rinse
Simulated residential exposure room (72 ± 4°F; 45 ± 20% RH; 0.6 ± 0.1 air changes/hour	32" x 48" or 24" x 32" laminate pieces (WisonArt® Basic type #107)	Presaturated Wipes	DDAC (0.0199%) C_{14} ADBAC (0.0266%)	Wipe on	1	2 hours	None
		Rag Wipe	DDAC (0.0375%) C_{14} ADBAC (0.0125%)	Wipe on	1	2 hours	None

TABLE B.1.2. Study Site and Use Pattern.							
		Trigger Sprayer	DDAC (0.0375%) C_{14} ADBAC (0.0125%)	Spray on to run off	1	2 hours	None
		Aerosol Spray	DDAC (0.0945%) C_{14} ADBAC (0.126%)	Spray on to run off	1	2 hours	None

1 Final concentration of the test compounds of interest in the application solutions.

For the first type of application, a presaturated wipe was used to treat each laminate piece (32 x 48 inches) by wiping the section from top to bottom and from side to side with overlapping strokes. For the second and third types of applications, concentrated Lemon Quat was diluted to a ~1.5% aqueous solution (~15 mg/mL) and applied using either a rag wipe or a hand-held trigger sprayer. For the wipe application, the rag was saturated with the dilute solution and wrung out, and then used for wiping over the laminate piece (81 cm x 122 cm) from top to bottom and from side to side with overlapping strokes. For the trigger spray application, the laminate pieces (81 cm x 122 cm) were sprayed as evenly as possible from top to bottom and side to side until each section was wet enough for run off. For the fourth type of application, a ready-to-use aerosol spray was used to treat each piece of laminate (61 cm x 81 cm) by spraying as evenly as possible from top to bottom and side to side until each section was wet enough for run off.

Following each type of application, the laminate pieces were allowed to air dry for 2 hours prior to testing for compound deposition. The 2-hour drying time was selected as both DDAC and ADBAC are reported to be stable on surfaces for this duration, and the duration of time more than satisfies the label-required contact time for surface disinfection.

Following drying, a single 10 cm x 10 cm section on each laminate surface was wiped to determine deposition of DDAC and C_{14} ADBAC. For wiping, a stainless steel template with a 10 x 10 cm cut out was randomly placed over a section and wiped first with a dressing sponge moistened with water (5 mL) and then with two dressing sponges moistened with 50% IPA in water (5 mL/sponge). The template was rinsed with IPA and dried between samples.

B.2. Sample Handling and Preparation

Following each wipe, the dressing sponges for the water and 50% IPA wipes were placed in separate glass jars and stored frozen ($\leq$-10°C) until analysis.

B.3. Analytical Methodology

Dressing sponge samples were analyzed for residues of DDAC and C_{14} ADBAC using an LC/MS/MS method (Method GPL-MTH-056). This method was previously validated for the recovery of DDAC and ADBAC (C_{12}, C_{14} and C_{16}) from moistened dressing sponges. A detailed description of the method and results from the method validation are reported in MRID# 46870702.

For this method, residues of DDAC and ADBAC are extracted by shaking the dressing sponges with acetonitrile/water/formic acid (70:30:0.016, v:v). Aliquots of the extracts are filtered and diluted, and deuterated internal standards are added for each analyte. Residues are then analyzed by LC/MS/MS using a C18 column and a mobile phase gradient of water to acetonitrile, each containing 0.2% formic acid. A single ion transition is monitored for each analyte, and internal standards are used for quantitation of each compound. The validated LOQ for DDAC and C_{14} ADBAC is 5.29 and 1.95 µg/sample for dressing sponges.

The above method was also validated in conjunction with the analysis of study samples. Triplicate control samples of dressing sponges were separately with each of the representative analytes at ~1x and 20x the LOQ.

C. RESULTS AND DISCUSSION

The LC/MS/MS method used to analyze dressing sponge samples was adequately validated in conjunction with the analysis of study samples. The recoveries of both analytes from triplicate samples of fortified dressing sponges are presented in Table C.1. Acceptable recoveries (91-109%) were obtained for both analytes at all fortification levels. The average recovery (with standard deviation) was 97.4 ± 5 for DDAC and 105 ± 4 for C_{14} ADBAC. Apparent residues of DDAC and C_{14} ADBAC were each <LOQ on control samples of dressing sponges.

Samples were stored frozen at ≤-10°C, except for when thawed for analysis (Table C.2). As samples were analyzed within 7 days of collection, supporting storage stability data are not required for these samples.

TABLE C.1. Summary of Concurrent Recoveries of DDAC and C_{14} ADBAC from Dressing Sponges.

Analyte	Spike Level (µg/sample)	Sample Size (n)	Recoveries (%)	Mean ± Std. Dev. (%)
DDAC	5.29	3	104, 99.2, 102	97.4 ± 5
	104	3	107, 108, 109	
C_{14} ADBAC	1.95	3	91.3, 98.5, 94.9	105 ± 4
	38.6	3	93.8, 102, 104	

TABLE C.2. Summary of Storage Conditions.

Matrix	Storage Temperature (°C)	Actual Storage Duration (days)	Interval of Demonstrated Storage Stability (days)
Dressing Sponges	<-10	7	N/A

The overall recovery from fortified laminate using the wiping method was 83.5% for DDAC and 76.5% for C_{14} ADBAC. These recovery values were used to correct measured residues from the main study in order to determine actual surface residues. The surface wiping method use for recovering dried residues of DDAC and C_{14} ADBAC was also adequately validated. Recoveries of both compounds were consistent from the four fortified laminate sections (Table C.3). The initial water wipe recovered 74.2 ± 3% of the applied DDAC and 68.2 ± 5% of the applied C_{14} ADBAC, and the 50% IPA wipes recovered 9.3 ± 7% of the applied DDAC and 8.3 ± 6% of the applied C_{14} ADBAC.

TABLE C.3.	**Removal Efficiency of DDAC and ADBAC from Treated Laminate Surfaces Using Moistened Dressing Sponges.**				
Wiping Solution	Fortification Level (μg/sample)	Sample Size (n)	Measured residues (ng/mL)	Recovery (%)	Mean ± Std. Dev. (%)
DDAC					
Water (1st wash)	65.6	4	50.6, 46.4, 49.2, 48.5	77.1, 70.7, 75.0, 73.9	74.2 ±3
50% IPA (2nd wash)	65.6	4	4.53, 12.8, 3.40, 3.61	6.91, 19.5, 5.18, 5.50	9.3 ± 7
Combined wipes	65.6	4	55.4, 59.2, 52.6, 52.1	84.5, 90.2, 80.2, 79.4	83.5
C_{14} **ADBAC**					
Water (1st wash)	24.4	4	17.5, 14.9, 17.0, 17.1	71.7, 61.1, 69.7, 70.1	68.2 ± 5
50% IPA (2nd wash)	24.4	4	1.39, 4.24, 1.30, 1.16	5.70, 17.4, 5.33, 4.75	8.3 ± 6
Combined wipes	24.4	4	18.9, 19.1, 18.3, 18.3	77.5, 78.3, 75.0, 75.0	76.5

Measured residues of DDAC and C_{14} ADBAC from the four replicate pieces of laminate treated by the four different methods are presented in Table C.4, and the relative recoveries of the residues by the water wipe and 50% IPA wipes are presented in Table C.5. The initial water wipe recovered the majority of residues for both compounds from each type of application. For the wipe-on type applications, the percent of recovered residues in the water saturated dressing sponges averaged 51-53% for C_{14} ADBAC and 55-65% for DDAC. For the two sprayer type applications, the percent of recovered residues in the water saturated dressing sponges averaged 68-71% for C_{14} ADBAC and 73-78% for DDAC. The differences in the percent of water dislodgeable residues between the wipe-on and sprayer type applications most likely reflects the higher residues levels initially deposited by the sprayer type applications. Comparing the two applications that used the same dilute solution of Lemon Quat, the levels of DDAC and C_{14} ADBAC were respectively 17x and 20x higher for the trigger sprayer application than for the rag wipe application. If the initial water wipe is assumed to approximate a surface rinse with water, and the recovery for the water wipes is adjusted to account for the overall recovery of both DDAC and C_{14} ADBAC by the surface wipe method, then these data indicate that a water rinse should remove approximately 52% of ADBAC and 60% of DDAC residues deposited from a wipe-on type application, and 70% of ADBAC and 76% of DDAC residues deposited by a sprayer type application. The most conservative value/assumption that should be used for risk assessment is 52% for ADBAC and 60% for DDAC.

TABLE C.4.	**Percentage of Quats Removed Using a Potable Water Rinse (PWR)**							
			Recovered Residues (µg/cm²) [1]					
Application Type	Analyte	Wipe Fraction	Samples				Average	Avg. quats removed using PWR (%)
C_{14} ADBAC	Pre-Saturated Wipes	Water Alone	0.0198	0.0195	0.0128	0.0111	0.0158	**52%**
		Total[2]	0.0272	0.0274	0.0197	0.0167	0.0228	
		Total Corrected[3]	0.0356	0.0358	0.0258	0.0218	0.0298	
		% removed[4]	56	54	50	51	53	
	Rag Wipes	Water Alone	0.0081	0.0097	0.0168	0.0205	0.0138	
		Total[2]	0.0139	0.0169	0.0235	0.0287	0.0208	
		Total Corrected[3]	0.0180	0.0221	0.0307	0.0375	0.0272	
		% removed[4]	45	44	55	55	51	
DDAC	Pre-Saturated Wipes	Water Alone	0.0155	0.0150	0.0092	0.0075	0.0118	**60%**
		Total[2]	0.0191	0.0183	0.0129	0.0100	0.0151	
		Total Corrected[3]	0.0229	0.0219	0.0154	0.0120	0.0181	
		% removed[4]	68	68	60	63	65	
	Rag Wipes	Water Alone	0.0284	0.0335	0.0528	0.0553	0.0425	
		Total[2]	0.0464	0.0578	0.0713	0.0814	0.0642	
		Total Corrected[3]	0.0556	0.0692	0.0854	0.0975	0.0769	
		% removed[4]	51	48	62	57	55	
C_{14} ADBAC	Trigger Spray	Water Alone	0.588	0.352	0.233	0.296	0.367	70%
		Total[2]	0.642	0.396	0.269	0.332	0.410	
		Total Corrected[3]	0.839	0.518	0.352	0.434	0.536	
		% removed[4]	70	68	66	68	68	
	Aerosol Spray	Water Alone	3.73	3.18	3.54	4.49	3.74	
		Total[2]	4.03	3.31	3.86	4.82	4.01	
		Total Corrected[3]	5.27	4.33	5.05	6.30	5.24	
		% removed[4]	71	73	70	71	71	
DDAC	Trigger Spray	Water Alone	1.46	0.867	0.615	0.886	0.957	76%
		Total[2]	1.61	1.00	0.727	1.01	1.09	
		Total Corrected[3]	1.93	1.20	0.871	1.21	1.31	
		% removed[4]	76	72	71	73	73	
	Aerosol Spray	Water Alone	3.00	2.71	3.02	4.01	3.19	
		Total[2]	3.25	2.83	3.29	4.32	3.42	
		Total Corrected[3]	3.89	3.39	3.94	5.17	4.10	
		% removed[4]	77	80	78	78	78	

1 Residues as determined by the analytical method.

2 Total residues from both water and 50% IPA wipes as determined by the analytical method (see MRID# 46870704).

3 Actual surface residues were calculated by correcting for the recovery of C14 ADBAC (76.5%) and DDAC (83.5%) using the surface wiping method (i.e. $\text{Total corrected}_{ADBAC} = \frac{\text{total x 100}}{76.5}$; $\text{Total corrected}_{DDAC} = \frac{\text{total x 100}}{83.5}$)

4 The % removed reflects the corrected recovery of C_{14} ADBAC and DDAC (i.e. % removed = water alone ÷ total corrected)
Bold denotes the % of ADBAC and DDAC residues removed.

TABLE C.5.	**Percent of Recovered Residues in Water and 50% IPA/Water Wipes.**							
Application Type	Analyte	Wipe Fraction	% of Total Recovered Residues					
			Samples				Average	S.D.
Pre-Saturated Wipes	C_{14} ADBAC	Water	72.8	71.2	65.0	66.5	68.9	3.7
		50% IPA/water	27.2	28.8	35.0	33.5	31.1	3.7
	DDAC	Water	81.2	82.0	71.3	75.0	77.4	5.1
		50% IPA/water	18.8	18.0	29.5	25.0	22.8	5.4
Rag Wipe	C_{14} ADBAC	Water	58.3	57.4	71.5	71.4	64.6	7.9

TABLE C.5. Percent of Recovered Residues in Water and 50% IPA/Water Wipes.

Application Type	Analyte	Wipe Fraction	% of Total Recovered Residues					
			Samples				Average	S.D.
		50% IPA/water	41.7	43.2	28.5	28.6	35.5	8.1
	DDAC	Water	61.2	58.0	74.1	67.9	65.3	7.2
		50% IPA/water	38.8	42.0	25.9	32.1	34.7	7.2
Trigger Spray	C_{14} ADBAC	Water	91.6	88.9	86.6	89.2	89.1	2.0
		50% IPA/water	8.4	11.2	13.2	10.8	10.9	2.0
	DDAC	Water	90.7	86.7	84.6	87.7	87.4	2.5
		50% IPA/water	9.6	13.6	15.4	12.0	12.6	2.5
Aerosol Spray	C_{14} ADBAC	Water	92.6	96.1	91.7	93.2	93.4	1.9
		50% IPA/water	7.5	3.8	8.4	6.9	6.6	2.0
	DDAC	Water	92.3	95.8	91.8	92.8	93.2	1.8
		50% IPA	7.8	4.2	8.3	7.2	6.9	1.8

D. CONCLUSIONS

This study is adequate for the purposes for which it was intended. The preliminary test indicated that dried residues of the representative quat compound DDAC and C_{14} ADBAC could be quantitatively recovered from a dried laminate surface by wipe with dressing sponges moistened with water and then 50% IPA. The data from the main study using the different application methods indicates that the amount of deposition of the two quat compounds was similar for a given type of application, but differed between the four application methods. Residues of both DDAC and C_{14} ADBAC resulting from the sprayer type applications were ~20x higher than from a rag wipe with a dilute solution and ~40x higher than an application using presaturated wipes.

In addition, the amounts of DDAC and C_{14} ADBAC residues recovered in the dressing sponges moistened with water indicate that a water rinse of a treated laminate surface should remove approximately 52% of ADBAC and 60% of DDAC residues deposited from a wipe-on type application, and 70% of ADBAC and 76% of DDAC residues deposited by a sprayer type application. The most conservative value/assumption that should be used for risk assessment is 52% for ADBAC and 60% for DDAC.

E. REFERENCES

None